Remarks

Claims 1-7, 13-17, and 19-24 were pending in the subject application. By this Amendment, claims 1-7, 13-17, and 19-24 have been canceled and new claims 25-55 have been added. The undersigned avers that no new matter is introduced by this amendment. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 25-55 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Claims 2-5, 13-17, and 20-24 have been rejected under 35 U.S.C. §112, second paragraph, as indefinite. The applicants respectfully submit that claims 2-5, 13-17, and 20-24 are not indefinite. However, by this Amendment, the applicants have canceled and rewritten claims 2-5, 13-17, and 20-24 as new claims 25-55. The applicants have addressed each of the points raised in the Office Action in turn, in the paragraphs that follow.

Support for claims 25-55 can be found throughout the subject specification and claims as originally filed. For example, support for claims 26 and 27 can be found at page 14, lines 1-14, and Figures 1-3 of the specification. Support for claims 37 and 38 can be found in Figures 1-3 and 6 of the specification.

The Office Action indicates that the recitation "said engineered Sertoli tissue" lacks antecedent basis. By this Amendment, the applicants have canceled claim 2, rendering this rejection moot. New claims 25-55 do not recite "said engineered Sertoli tissue."

The Office Action indicates that method claims 13-16 do not recite any affirmative steps. Claim 13 recites the step of "co-culturing Sertoli cells and therapeutic cells" and claim 14 recites the step of "segregating the Sertoli cells away from the therapeutic cells", which the applicants submit are affirmative steps that do not render the method claims indefinite. However, by this Amendment, the applicants have canceled and rewritten claims 13-16 as new claims 40-45, in order to lend greater clarity to the claimed subject matter. Support for new claims 40-45, can be found, for example, at page 12, lines 8-13, page 13, lines 9-12, page 14, lines 1-14, and in claims 21-23 of the subject application as originally filed. Support for claims 40-45 can also be found, for example, at page 18, line 8 and lines 17-19, and page 19, lines 10-30. Claim 40 recites the steps of co-culturing Sertoli

cells and non-Sertoli cells, and organizing the Sertoli cells and the non-Sertoli cells such that the Sertoli cells form an outer wall around the non-Sertoli cells, and the non-Sertoli cells are contained within the lumen. Support for the organization of Sertoli cells and non-Sertoli cells can be found, for example, at page 12, lines 9-13, and lines 28-30 of the subject specification, which indicates that the cells contained within the lumen of the biochamber are different than the facilitator cells, which are preferably Sertoli cells. Support for claim 45 can be found, for example, at page 14, lines 1-5, and Examples 1 and 2 in the specification.

The Office Action indicates that there is insufficient antecedent basis for the phrase "the step of segregating" in claim 14. Claim 14 has been canceled, rendering this rejection moot. Claim 14 has been rewritten as new claim 41, which introduces the segregation step. Antecedent basis for "Sertoli cells" and "non-Sertoli cells" is found in claim 40.

The Office Action indicates that there is insufficient antecedent basis for the term "the facilitator cells". The applicants have canceled claim 15, rendering this rejection moot. Claim 15 has been rewritten as new claim 42, which does not recite the term "the facilitator cells".

The Office Action indicates that, based on claim 17, it is unclear what material forms the biochamber. As indicated above, by this Amendment, the applicants have canceled and rewritten claim 17 as new claim 50. Claim 50 recites that the biochamber is formed with an outer wall of Sertoli cells, which defines the lumen; thus, the materials forming the biochamber are recited.

The Office Action indicates that, based on claims 1, 20, and 21, it is unclear if it is intended that three different tissue structures are claimed. As indicated above, claims 1, 20, and 21 have been canceled, rendering this rejection moot. In order to lend greater clarity to the claimed subject matter, claims 1, 20, and 21 have been rewritten as new claims 25 and 39, which are directed to biochambers of the subject invention.

In view of the above remarks and amendments to the claims, the applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

Claims 1-7, 13, 16, 19, and 20-24 have been rejected under 35 U.S.C. §112, first paragraph, as lacking sufficient written description. As indicated in the preceding paragraphs, in order to lend greater clarity to the claimed subject matter, claims 1-7, 13, 16, 19, and 20-24 have been canceled and rewritten as new claims 25-55. Page 5 of the Office Action indicates that Figures 1-3 of the

subject specification are diagrams of Sertoli cell behavior, and a lumen surrounded by an outer wall of Sertoli cells is shown. The Office Action further indicates, however, that Figures 4-7 are photographs showing Sertoli cell-islet cell structures and Sertoli cell-neuron structures in which a lumen is not apparent. At page 6, the Office Action indicates that "evidently, there are discrepancies between the diagrams and histological photographs of tissue constructs and among disclosed and claimed structures in the specification."

The applicants respectfully submit that there is no discrepancy or inconsistency between the Figures and the claimed invention. As indicated at page 11 of the subject specification, and as indicated within the captions of the figures, Figures 1-3 show biochambers of the subject invention in which each biochamber has a lumen that is defined by an outer wall of Sertoli cells, as recited in the currently pending claims. Specifically, as indicated at page 11 of the specification, Figure 1 is a diagram showing formation of a biochamber of the subject invention on a substrate, Figure 2 shows a comparison of biochambers of the subject invention produced by conventional culture and microgravity culture, and Figure 3 shows a biochamber of the subject invention, including a proposed mechanism for the immunoprotective effect the wall of Sertoli cells has on the cells contained within the lumen. The insets of Figures 2 and 3 also show micrographs of the sectioned biochambers of the subject invention, with the outer wall of Sertoli cells and lumen containing islet cells evident.

Figures 4 and 5 are photographs showing Sertoli cell-islet cell constructs grown in conventional co-culture, demonstrating continued production of insulin by the islet cells in co-culture. Although Figures 4 and 5 show Sertoli cell-islet cell constructs, Figures 4 and 5 are not intended to depict the biochamber constructs of the claimed invention, which include an outer wall of Sertoli cells which defines a lumen. In contrast, Figure 6 is a photograph of biochambers of the subject invention. The applicants respectfully submit that, because Figure 6 represents a photograph of biochambers as a "whole mount", one of ordinary skill in the art would not expect to be able to observe the interior compartment or lumen of the biochamber, because it is obscured by surrounding external features. Therefore, only the outer walls of the biochambers are readily visible in Figure 6. Likewise, Figure 7 shows two micrographs of sections of biochambers of the subject invention, which are immunostained for FasL (a Sertoli cell marker) and neuron-specific marker. The lumen of

the biochamber and the structure of the defining Sertoli cell wall, which is somewhat distorted due to sectioning, are readily apparent in the FasL-stained section (left side of Figure 7). Therefore, the applicants respectfully submit that the subject specification, including Figures 1-7, demonstrate that the applicants were in possession of the claimed invention, as required by 35 U.S.C. §112, first paragraph.

In view of the remarks above and the amendments to the claims, the applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Claims 1-7, 13-17, and 19-24 have been rejected under 35 U.S.C. §112, first paragraph, as non-enabled by the subject specification. At page 9, the Office Action indicates that the claims are drawn to "several biochambers with different structures, compounds, and protective cells," but the specification fails to provide an adequate description for the biochambers, compounds, and cells encompassed by the claims. As indicated in the preceding paragraphs, the applicants have amended claims 1-7, 13-17, and 19-24 to lend greater clarity to the claimed subject matter. The applicants respectfully submit that the currently pending claims are fully enabled by the subject specification.

At pages 10 and 11, the Office Action cites the Willing *et al.* publication and indicates that, "it is unclear whether xenotransplantation could be tolerated in the presence of Sertoli cells, whether Sertoli cells could survive a period long enough to achieve therapeutic effect in humans, and whether the technique could be used for all types of diseases". The applicants have addressed each of these issues in the paragraphs that follow.

The applicants respectfully submit that the Willing *et al.* publication actually supports the fact that the currently pending claims are enabled by the subject specification. For example, the Willing *et al.* publication describes experimental animal research data and suggests that cellular therapy using Sertoli cells "might turn out to be one of the most versatile, novel and 'precedent setting' approaches to cell transplantation in the CNS to date" (page 476, last sentence). At page 472 of the Willing *et al.* publication, it is indicated that Sertoli cells do not express major histocompatibility (MHC) proteins I or II; therefore, the applicants respectfully submit that one of ordinary skill in the art would expect that it is unlikely that the immune system of any species (including humans) would detect the presence of Sertoli cells, regardless of the species from which the Sertoli cells are derived. There is nothing in the Willing *et al.* publication that suggests that Sertoli cell co-transplants would

be unviable in a human. Rather, the Willing *et al.* publication merely indicates that experimentation regarding the viability of Sertoli cells and transplantable cells in humans had not yet been attempted at the time of publication (page 475, second column, lines 8-17, and page 476, first column, lines 8-18).

As indicated at page 15 of the subject specification, "Sertoli cells are terminally differentiated, and the cells are mitotically inactive. They live for a long period of time, and potentially as long as any therapeutic cell type that can be engineered into the Sertoli cell biochamber." In addition, submitted herewith is a copy of the Luca *et al.* publication (*AAPS PharmSciTech.*, 2(3), article 15, 2001), which describes co-microencapsulation of Sertoli cells with islets in alginate/poly-L-ornithine, and implantation of the microcapsules in mice, which resulted in improved functional performance of the islets. Furthermore, as shown in Figure 6 of the Luca *et al.* publication, the presence of Sertoli cells actually increased the survival of the islet grafts post transplantation for a period longer than eighty days.

The biochambers of the claimed invention can be constructed to contain non-Sertoli cells of potentially any cell type. Therefore, the applicants submit that the biochambers of the claimed invention could be used to treat any disease for which cell transplantation may alleviate symptoms resulting from the diseased or damaged cells in the body, for which the transplanted cells are replacing or compensating. For example, at page 476, paragraph bridging the left and right columns, the Willing *et al.* publication itself indicates that "the immunosuppressive effects of the [Sertoli] cell will be useful in the treatment of any disorder where transplantation has been shown to be a viable option" (emphasis added). Examples of transplant cells and pathological conditions for which such cell therapy would be effective include, but are not limited to, pancreatic beta cells (type I diabetes), dopaminergic cells (Parkinson's disease), Factor VIII producing cells (hemophilia), neurons (stroke), cardiac muscle cells (myocardial infarction), adrenal cortex cells (Addison's disease and Cushing's disease), and gastrointestinal endocrine cells (Krone's disease). In particular, page 476, second column, of the Willing *et al.* publication indicates that the "[Sertoli] cell's potential for treating neurodegenerative diseases is great." Therefore, the applicants respectfully submit that the claimed invention is enabled by the subject specification.

In view of the remarks above and the amendments to the claims, the applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Claims 13, 14, and 19 are rejected under 35 U.S.C. §102(a) as being anticipated by Korbutt *et al.* (*Diabetologia*, 2000, 43:474-480). The Office Action indicates that the Korbutt *et al.* publication describes a method of co-culturing Sertoli cells with an islet cell preparation before transplantation to allow Sertoli cells to aggregate and surround the islet cells. The applicants respectfully submit that the Korbutt *et al.* publication does not teach or suggest the applicants' claimed invention. However, as indicated in the preceding paragraphs, claims 13, 14, and 19 have been canceled and rewritten to lend greater clarity to the claimed subject matter, rendering this rejection moot.

New claims 28 and 39 are directed to a biochamber including an outer wall of Sertoli cells which defines a lumen containing a plurality of non-Sertoli cells, where the Sertoli cells encapsulate the plurality of non-Sertoli cells. New claim 40 is directed to a method of making a biochamber by co-culturing Sertoli cells and non-Sertoli cells, and organizing the Sertoli cells and the non-Sertoli cells, such that the Sertoli cells form an outer wall defining a lumen, where the non-Sertoli cells are contained within the lumen. The applicants respectfully submit that the Korbutt *et al.* publication does not disclose a biochamber having an outer wall of Sertoli cells defining a lumen. These structures are described in the subject specification at page 12, lines 15-30, page 13, lines 1-4, and page 14, lines 1-14, for example.

As indicated at page 478, Figures 2C and 2D of the Korbutt *et al.* publication describe Sertoli cell-islet cell grafts, where clusters of Sertoli cells are shown in the vicinity of the immunostained islet cells. As indicated in the Materials and Methods section at page 475 of the Korbutt *et al.* publication, these Sertoli cell-islet cell grafts were prepared by culturing the Sertoli cells and islet cells separately and consecutively aspirating islet cells and Sertoli cells into polyethylene tubing and pelleting the cells by centrifugation. Therefore, the Sertoli cell-islet cell grafts described in the Korbutt *et al.* publication represent random distributions of the two cell types, without any apparent organization. The Korbutt *et al.* publication does not describe or suggest a biochamber including an outer wall of Sertoli cells that defines a lumen, as currently claimed. As described at page 12, lines 15-21, and page 14, lines 1-14, of the subject specification, the biochambers of the subject invention are composed of discrete walls that define a lumen, which may contain non-Sertoli cells for

transplantation. The biochambers of the subject invention are designed to mimic the intraepithelial barrier observed in the testis and referred to as the blood-testes barrier. The Korbutt *et al.* publication does not describe co-culturing and organizing the Sertoli cells and islet cells such that the islet cells are contained within a lumen defined by an outer wall or barrier of Sertoli cells. At most, Figures 2C and 2D of the Korbutt *et al.* publication show random arrangements of islet cells that are discontinuously surrounded by Sertoli cells. The Korbutt *et al.* publication does not describe the highly organized structures of the subject invention.

As the Examiner is aware, in order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991). The applicants respectfully assert that the cited reference does not teach or suggest each and every element of the applicants' claimed invention. The Korbutt *et al.* publication does not teach or suggest a biochamber including an outer wall of Sertoli cells defining a lumen, or methods of making such biochambers. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(a) is respectfully requested.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

Glenn P. Ladwig

Glenn P. Ladwig
Patent Attorney
Registration No. 46,853
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: Saliwanchik, Lloyd & Saliwanchik
A Professional Association
2421 NW 41st Street, Suite A-1
Gainesville, FL 32606-6669

GPL/mv

Attachments: Luca *et al.* publication
Amendment Transmittal Letter